

# Supporting Information

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## SI Materials and Methods

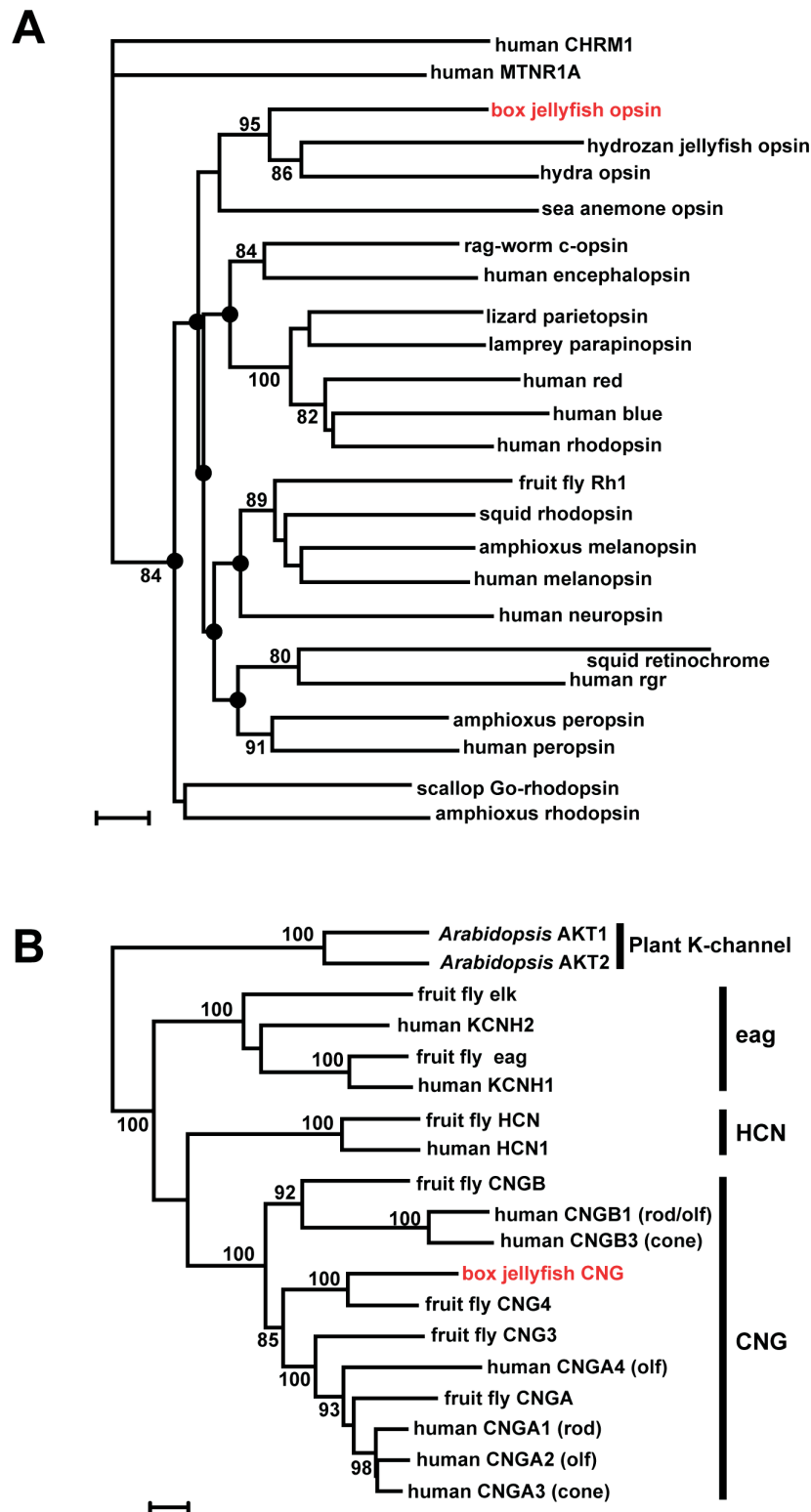
**Amino Acid Sequences.** The accession numbers of amino acid sequences used for analyses are as follows: Box jellyfish opsin, AB435549; sea anemone opsin, BR000662; hydra opsin, Contig39347:487820–488855 (<http://hydrazome.metazome.net>); hydrozoan jellyfish opsin, AB332435; human encephalopsin, AF140242; ragworm c-opsin, AY692353; human rhodopsin, U49742; human blue, M13299; human red, Z68193; lamprey parapinopsin, AB116380; lizard parietopsin, DQ100320; amphioxus peropsin, AB050610; human peropsin, AF012270; human rgr, U15790; squid retinochrome, X57143; human melanopsin, AF147788; amphioxus melanopsin, AB205400; squid rhodopsin, X70498; fruit fly Rh1, K02315; human neuropsin, AY377391; amphioxus rhodopsin, AB050606; scallop G<sub>o</sub>-rhodopsin, AB006455; human muscarinic acetylcholine receptor M1 (CHRM1), NM\_000738; human melatonin receptor 1A (MTNR1A), NM\_005958; *Arabidopsis* AKT1, U06745; *Arabidopsis* AKT2, U40154; fruit fly elk, U04246; human KCNH2, BC001914; fruit fly eag, M61157; human KCNH1, NM\_002238; fruit fly HCN, AF124300; human HCN1, NM\_021072; fruit fly

CNGB, NM\_137763; human CNGB1, NM\_001297; human CNGB3, NM\_019098; box jellyfish CNG, AB435552; fruit fly CNG4, NM\_167441; fruit fly CNG3, NM\_137871; human CNGA4, NM\_001037329; fruit fly CNGA, NM\_057768; human CNGA1, NM\_000087; human CNGA2, NM\_005140; human CNGA3, NM\_001298.

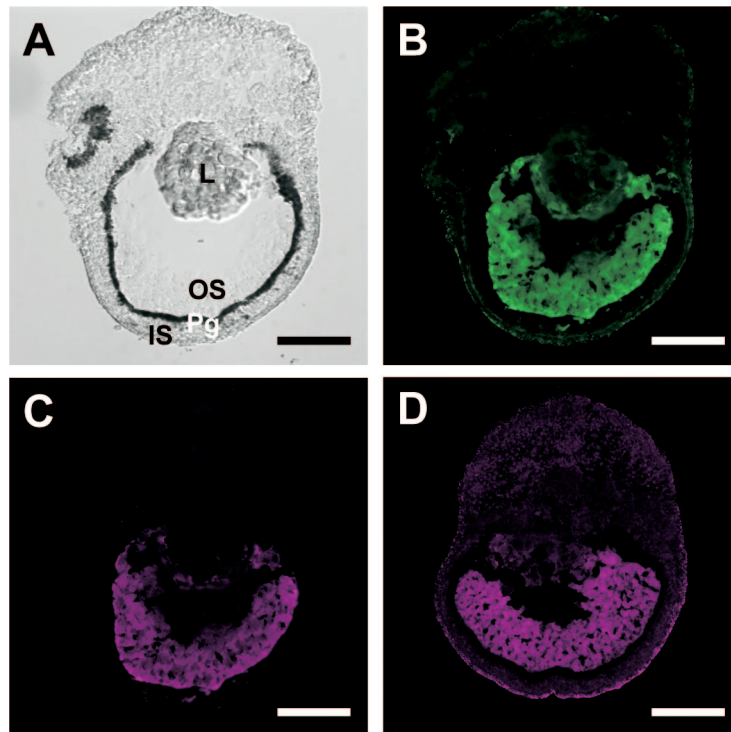
**Western Blot Analysis.** The proteins extracted from half a rhopalia were separated by 12% SDS/PAGE, transferred onto a PVDF membrane, and incubated with 1:500 diluted anti-box jellyfish opsin antiserum. Visualization was carried out by the ABC method (Vectastain) and by staining with 3,3'-diaminobenzidine (Sigma).

**In Situ Hybridization.** Digoxigenin-labeled antisense and sense RNA probes for the box jellyfish opsin were synthesized by using the DIG RNA labeling kit (Roche). Sections were pretreated with proteinase K and hybridized with each RNA probe. The probe on the sections was detected with alkaline phosphatase-conjugated anti-digoxigenin (Roche) by using a blue 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium color reaction.

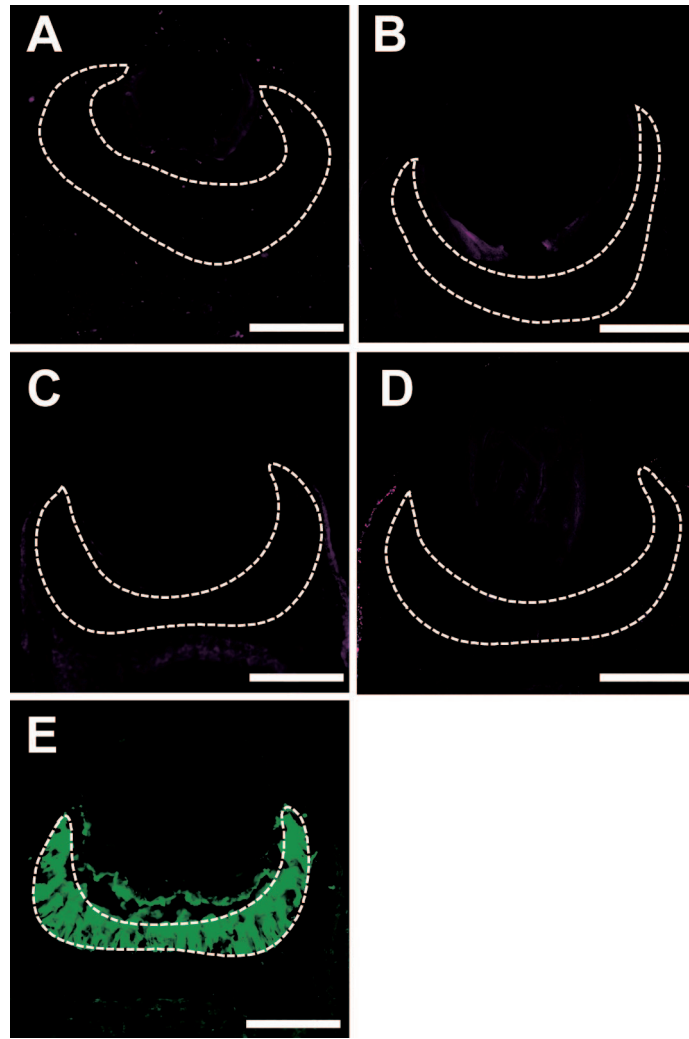




**Fig. S2.** Molecular phylogenetic trees of the opsin family (A) and cyclic nucleotide-gated channel family (B). (A) The amino acid sequences of opsins that were revealed to function as photopigments and their apparent homologs were included in the phylogenetic analysis. The human CHRM1 and MTNR1A were used as an outgroup. The relationships among subfamilies (black circles) are ambiguous. See Fig. 4. (B) Plant K-channels were used as an outgroup. The subfamily names of animal cyclic nucleotide-gated channels are shown on the right side of each cluster. eag, ether-a-gogo; HCN, hyperpolarization-activated and cyclic nucleotide-gated; CNG, cyclic nucleotide-gated. Box jellyfish sequences are indicated by red letters. Bootstrap probabilities higher than 80% are indicated at each branch node. (Scale bars: 0.1 substitutions per site.)



**Fig. S3.** Opsin- $G_{\alpha_5}$ -adenylyl cyclase cascade in the small lens eye of the box jellyfish. (A) Nomarski image of the small lens eye of the box jellyfish. L, lens; OS, outer segment; Pg, pigment granule-rich region; IS, inner segment. (B-D) Immunofluorescence labeling of outer segments of box jellyfish visual cells with the anti-box jellyfish opsin antibody (B, green), the anti- $G_{\alpha_5}$  antibody (C, magenta), and anti-adenylyl cyclase antibody (D, magenta). (Scale bars: 100  $\mu\text{m}$ .)



**Fig. S4.** Immunohistochemical analyses of the box jellyfish lens eye using  $G\alpha_t$  (A),  $G\alpha_o$  (B),  $G\alpha_q$  (C) and  $G\alpha_{12}$  (D). Fluorescent signals derived from these G proteins are not observed in the outer segments of visual cells (dotted traces) where the jellyfish opsin is localized (E). (Scale bars: 100  $\mu\text{m}$ .)